CLUTCH AND BODY SIZE ANALYSIS OF *SPHAEROTHECA ROLANDAE* (ANURA: RANIDAE)[1]

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Clutch size analysis of *Sphaerotheca rolandae* was examined based on eggs obtained from ovaries of females and laid by amplecting females. Clutches of 42 amplecting females were counted. The measurement of snout-vent length (SVL) and weight (WT) of amplecting males and females indicate larger females. The minimum and maximum clutch size of 42 females was 449 and 1037 respectively; there was no correlation between body size and clutch size. Eggs counted from both the ovaries of 18 gravid females suggest that the minimum and maximum clutch size is 1896 and 5472 respectively. Like in amplecting females, the body size is not a factor of clutch size in gravid females. Larger egg counts for gravid females than for amplecting females were due to the presence of immature oocytes in the ovaries of the former.

Key words: Anura, Ranidae, *Sphaerotheca rolandae*, clutch

INTRODUCTION

Reproductive pattern and clutch size of amphibians are relatively well documented for temperate species than tropical ones (Tilley 1968; Bruce 1969; Salthe 1969; Crump 1974; Brockelman 1975). A good deal of information is also available on the annual reproduction of tropical anurans from Southeast Asia (i.e. *Bufo melanostictus* in Java: Church 1960a and in Singapore: Berry 1964; *Rana cancrivora* in Java: Church 1960b; *Rana erythraea* in Borneo: Inger and Greenberg 1963; *Kaloula pulchra*, *Microhyla butleri*, *M. heymonsi* and *Leptobrachium nigrops* in Singapore: Berry 1964; *Limnonectes leporinus*, *R. macrodon* (incertae sedis), *Limnonectes ibanorum* and *R. hosii* in Borneo: Inger and Bacon 1968).

A series of studies by Mohanty-Hejmadi and Dutta (1979); Dutta and Mohanty-Hejmadi (1976); Dutta *et al.* (1991, 1992); Mohanty-Hejmadi and Dutta (1988); Mohanty (1993) and Rath (1994) report the number of eggs per clutch of *Hoplobatrachus crassus*, *H. tigerinus*, *Limnonectes limnocharis* (= *Fejervarya syhadrensis*), *Euphlyctis cyanophlyctis*, *Ramanella variegata* and *Bufo melanostictus*. In this paper, morphometric parameters and clutch size of another member of Ranidae, *Sphaerotheca rolandae* has been described.

MATERIAL AND METHODS

Clutch sizes have been analyzed for both gravid and amplecting females. Amplecting males and females (42) were collected from breeding grounds at night over a period of nine years. They were brought to the laboratory in polythene bags and transferred to glass containers with amphibious environment. Precaution was taken to avoid external disturbances such as light and sound, which interfere with safe egg laying. Immediately after egg laying, the egg clutches were transferred to enamel trays containing conditioned tap water. The snout-vent length (SVL) and weight (WT) of each specimen was measured. The eggs were counted and reared in the laboratory.

To determine the clutch size of gravid females, specimens were collected from their breeding grounds and preserved in 4% formaldehyde. Prior to preservation, they were measured and weighed. A total of 18 gravid females were collected during four years. The preserved specimens were dissected and both the ovaries were removed. The eggs were separated from lobules and staged following Dumont (1972). At least 20 eggs (containing all the available stages) were measured from each clutch to determine the range of ovum size.

Cumulative means, SD, minimum and maximum SVL and weight (WT) of amplecting males and females have been determined from individual parameters. Correlation matrix of SVL and WT of amplecting pairs and clutch sizes has been analysed. Year-wise analysis of morphometric parameters for both males and females, and clutch sizes has been conducted to provide a eomparative statement.

RESULTS

Clutch and body size of amplecting pairs (Tables 1-3): The minimum and maximum SVL and WT of amplecting

Table 1: Cumulative mean, Standard Deviation (SD), minimum and maximum of SVL (mm) and WT (gm) of amplecting males and females and clutch size (N = 42)

	Minimum	Maximum	Mean	SD
SVL (Male)	29.00	38.00	34.004	2.587
WT (Male)	3.80	5.00	4.397	0.435
SVL (Female)	35.00	44.00	39.866	2.192
WT (Female)	5.20	7.90	6.552	0.774
Clutch	449.00	1037.00	754.428	150.742

i. The 't' for SVL of amplecting males and females is 11.381, which is highly significant at 1% level of probability.

ii. Regression relationship between SVL (female) and clutch size. Clutch size = - 0.0463 + 756.1082.

Table 2: Correlation matrix of SVL (mm) and WT (gm) of amplecting males and females and clutch size (refer to Table 1)

	SVL (male)	WT (male)	SVL (female)	WT (female)	Clutch
SVL male	1.000				
WT male	0.748*	1.000			
SVL female	-0.054 NS	-0.038 NS	1.000		
WT female	-0.084 NS	-0.116 NS	0.828**	1.000	
Clutch	0.116	0.298 NS	-0.001	0.058 NS	1.000

NS = Not significant.

* = Significant at 5% level.

** = Significant at 1% level.

females and males are presented in Table 1. Correlation matrix of SVL and WT is presented in Table 2.

Year-wise analysis (Table 3) indicated that females with lowest SVL (35.74 mm) were observed in 1987 and highest (43 mm) in 1992. Males with lowest SVL (30.96 mm) and highest SVL (35.66 mm) were recorded in 1986 and 1991, respectively. Analysis of weight showed the lowest (5.46 gm) and heaviest (7.56 gm) females in 1987 and 1991, respectively. It was interesting to note that the weight of the males was comparable with SVL, as the minimum (3.90) and maximum (4.73) were observed in 1986 and 1991, respectively. The minimum value for mean number of eggs (646.63 ±177.220) was recorded in 1980, and the maximum value for mean number of eggs (918.5 ±19.091) in 1992. This indicated that there was no correlation between mean clutch size and mean SVL or WT of amplecting males and females, because neither males nor females with minimum or maximum mean SVL and WT were recorded during 1980 or 1992.

The mean clutch size of amplecting males and females is 754.428 ±150.742, the minimum clutch size (449) was for the female with 38.0 mm SVL and the maximum clutch size (1037) was for the female with 39.0 mm SVL. However, the smallest female (35.0 mm SVL) laid 820 eggs and the largest female (44.0 mm SVL) laid 932 eggs. This indicates that no correlation exists between the body size and clutch size in this species.

A comparative assessment of SVL of amplecting males and females indicated that the smallest male (29.0 mm SVL) was found in amplexus with a female measuring 40.9 mm in SVL, and the largest male (38.0 mm SVL) amplected with a female measuring 38.0 mm in SVL. Therefore, no correlation exists between SVLs of amplecting males and females. However, all the amplecting males were smaller than the amplecting females. The "t" value for SVLs of amplecting males and females is 11.381, which is highly significant at 1% level of probability.

Clutch and body size of gravid females (Table 4): Interestingly, the largest female (50.0 mm SVL), with the maximum weight (9.3 gm), had the maximum number of oocytes (5472). However, the smallest female (39.0 mm SVL) had more number of oocytes (4048) than several larger females. Data on mean, SD and range of SVL, WT and clutch size is presented in Table 5. Correlation matrix of SVL, WT and clutch, size is given in Table 6.

Table 3: Year-wise mean and Standard Deviation (SD) of clutch size, SVL (mm) and WT (gm) of amplecting males and females

Year	No	SVL (M)	SD	SVL (F)	SD	WT (M)	SD	WT (F)	SD	Clutch	SD
1980	8	33.44	2.678	40.14	1.597	4.57	0.426	6.37	0.730	646.63	177.220
1981	8	35.0	2.035	39.31	1.251	4.64	0.534	6.37	0.561	755.37	206.230
1986	5	30.96	1.494	40.0	1.842	3.90	0.141	6.90	0.703	747.0	132.898
1987	5	34.6	3.111	35.74	0.622	4.40	0.514	5.46	0.151	748.0	102.924
1988	4	33.87	3.567	40.57	0.403	4.65	0.624	6.77	0.330	769.25	161.438
1990	3	34.53	0.503	39.46	1.331	4.23	0.057	5.93	0.513	783.33	158.295
1991	3	35.66	3.214	42.36	1.001	4.73	0.550	7.56	0.251	877.66	114.023
1992	2	32.40	1.272	43.0	1.414	4.10	0.141	7.50	0.565	918.50	19.091
1993	4	35.50	1.249	41.57	1.201	4.20	0.081	7.22	0.287	653.50	97.154

M: Male; F: Female

The clutch size of gravid females was more than that of the amplecting females. This is because the total oocytes present in both the ovaries of gravid females were taken into account. Also, the oocytes of gravid females were at stages I-III and VI, whereas for amplecting females, only mature ova (stages VI) were laid. The size (diameter) of the ovum also varied between the clutches of both the categories. The eggs laid by amplecting females were >1.0 mm in diameter, whereas the eggs of gravid females were <1.0 mm in diameter and in several cases, the egg sizes were <0.5 mm in diameter. This is obviously due to the presence of oocytes of Stage I, II and III in the ovaries.

DISCUSSION

Sphaerotheca rolandae is a seasonal breeder (Das 1995) and reproductive output of seasonal breeding depends only on the number of eggs laid by a female. Several workers (Terentjev 1960 on anurans; Salthe 1969 on salamanders; Matsui and Ota 1984 on anurans) have reported correlation between body and clutch size. Clutch size is generally affected by the mode of reproduction and aquatic species have larger clutch sizes than species dwelling on land (Crump 1974; Duellman and Trueb 1985). No correlation was observed between the body and clutch size of *S. rolandae* and this is also comparable with previous studies on *Hoplobatrachus tigerinus*, *H. crassus*, *Fejervarya syhadrensis* and *Polypepdates maculatus*. Terentjev (1960) first reported the correlation between body size and clutch size of frogs. Inger and Bacon (1968) tested the formula of Terentjev (1960) for studies on *Rana erythraea*, which was significantly below expectation. Terentjev's formula has not been used for clutch size analysis of *S. rolandae*. However, egg clutches of 42 females have been utilized to establish the correlation with SVL, WT and clutch sizes. The gravid specimens were utilized to assess the number of oocytes present in both the ovaries, where as the amplectant specimens were utilized to assess the number of eggs laid. Interestingly, the clutch sizes (ovarian eggs) of gravid females were more than the clutch size (laid eggs) of amplecting females. No such comparative assessment of clutch size of any Indian anuran is available. When the clutch and body size of *S. rolandae* is compared to that of other Indian ranids (*H. tigerinus*, *H. crassus* and *F. syhadrensis*), it is noticed that *H. tigerinus* and *H. crassus* (except *F. syhadrensis*) laid more eggs than *S. rolandae* and their sizes were also comparable with that of Inger and Bacon's (1968) studies on four ranids from Borneo. Hence, it is assumed that in ranids the clutch size increases with the increase of body size between species, but not within a species.

A comparative assessment of clutch sizes of *S. rolandae* with *P. maculatus*, a rhacophorid (Rath 1994) indicates a deviation from the assumption made for ranids i.e. more eggs for larger species. Though *P. maculatus* is a relatively larger species the clutch size of this species is small. This deviation

Table 4: SVL, WT, clutch size, oocyte stages and ovum sizes of gravid females

Year	Gravids	SVL (mm)	WT (gms)	Clutch size	Oocyte stages	Ovum size (mm) range
1989	1	42.0	7.5	2224	I, II, VI	0.391-1.609
	2	45.0	5.8	3176	I, II, VI	0.478-1.304
	3	45.0	5.5	2968	I, II, III, VI	0.478-1.391
	4	46.0	8.9	2912	I, VI	0.347-1.174
	5	45.0	7.5	2460	I, II, VI	0.348-1.131
	6	41.0	7.5	3935	I, VI	0.435-1.304
	7	40.0	7.0	2520	I, II, III, VI	0.478-1.261
	8	50.0	9.3	5472	I, VI	0.348-1.061
	9	44.0	5.2	1896	I, VI	0.478-1.174
	10	45.0	7.9	2846	I, VI	0.435-1.304
	11	39.0	7.3	4048	I, II, VI	0.565-1.261
1991	12	44.0	6.5	3665	I, VI	0.456-1.189
	13	40.0	5.0	2650	I, VI	0.535-1.125
	14	42.5	5.0	2455	I, VI	0.425-1.168
1992	15	43.5	5.2	2022	I, VI	0.478-1.311
	16	44.0	6.5	1950	I, VI	0.460-1.385
1993	17	41.5	5.0	3605	I, VI	0.475-1.235
	18	43.0	5.3	2232	I, VI	0.465-1.165

i. Mean clutch size is 2946.444

ii. Regression relationship between SVL (female) and clutch size: Clutch size = -1106.1250 + 93.461 SVL

Table 5: Mean, Standard Deviation (SD), minimum and maximum of SVL (mm), WT (gm) and clutch size of gravid females (refer to Table 4)

Name	Mean	SD	Minimum	Maximum
SVL	43.361	2.616	39.00	50.00
WT	6.550	1.381	5.00	9.30
Clutch	2946.444	917.628	1986.00	5472.00

Table 6: Correlation matrix of SVL (mm), WT (gm) and clutch size of gravid females (refer to Table 5)

	SVL	WT	Clutch
SVL	1.000		
WT	0.418 NS	1.000	
Clutch	0.266 NS	0.4978*	1.000

NS = Not Significant.
* = Significant at 5% level

could be attributed to the specialized breeding strategy of *P. maculatus*, which lays eggs inside foams and that the embryonic development is completed inside the foams. Thus, *P. maculatus* exhibits indirect parental care for successful reproduction. On the other hand, though *S. rolandae* lays a larger clutch, due to direct egg laying in water, the eggs are subjected to environmental constraints. Thus, the hatching success is reduced like in other ranids. It is interesting to note that all the species with larger body – larger clutch size, unlike *P. maculatus*, have no parental care.

Additional comparative assessment of clutch sizes of *S. rolandae* with other inter-generic species (microhylids: *Uperodon systoma*: (unpublished data) and *Ramanella variegata*: Dutta *et al.* 1992), also suggests variation. Being a larger species than *S. rolandae*, the clutch sizes of *U. systoma* are also larger than *rolandae*. Similarly, *R. variegata* a smaller species than *S. rolandae* has a smaller clutch size. Interestingly, all the above three species live sympatrically and are burrowers. Hence, it is hypothesized that specialized mode of living is also correlated with clutch size.

Several other studies (Kuramoto 1978; Inger and Bacon 1968; Wagner 1965, cited by Duellman and Trueb 1985; Mohanty-Hejmadi and Dutta 1979; Mohanty 1993) have also reported clutch sizes of both tropical and temperate species of ranids. The maximum clutch size of a ranid *R. fuscigula* (15,000 eggs) was reported by Wagner (1965). However, the maximum clutch size known for any anuran is 22,412 for *Bufo melanostictus* (unpublished). On the whole, the clutch size of ranids breeding seasonally and occupying similar habitats is correlated to body size at inter-generic level. Thus, the present study agrees with the interpretation of Salthe and Mecham (1974) who reported "female body size increases phylogenetically within any given mode of reproduction with increase in clutch size".

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